

# Experiences of the Janus Serum Bank in Norway

Egil Jellum, Aage Andersen, Per Lund-Larsen, Liv Theodorsen, and Harald Ørjasæter

The Norwegian Cancer Society, Oslo, Norway

The ongoing JANUS project was started in 1973. The serum bank comprises 424,938 serum samples consolidated from 293,692 donors. The specimens are stored at  $-25^{\circ}\text{C}$ . From 1 to 13 consecutive samples are available from each donor. Up to October 1993 about 14,000 of the donors had developed some form of cancer. Frozen serum samples collected from a few months to 19 years prior to clinical recognition of their disease are available for research purposes. The principle aim of the JANUS project is to search in the premorbid sera for chemical, biochemical, immunological or other changes that might be indicative of cancer development at early stages. Gas chromatography-mass spectrometry and two-dimensional protein electrophoresis have been used to evaluate the stability of the frozen sera. Some recent findings are: CA-125 may be elevated months prior to the diagnosis of ovarian cancer; serum thyroglobulin may be a preclinical tumor marker in subgroups of thyroid cancer; low levels of selenium in serum reflects increased risk of thyroid cancer; raised antibodies in serum against Epstein-Barr virus is a risk factor for development of Hodgkins disease; prostate-specific antigen may be elevated years prior to clinical diagnosis of prostate cancer; and linoleic acid in serum phospholipids is inversely related to breast cancer risk. The serum bank is, in principle, suitable for environmental studies, e.g., human exposure assessment. The steering committee of the JANUS project is open to suggestions for collaborative research on this topic. — *Environ Health Perspect* 103 (Suppl 3):85-88 (1995)

Key words: frozen sera, twenty-year collection, cancer information, prediagnostic sera, tumor markers, stability of sera, chromatography, electrophoresis, cancer and risk factors, environmental studies

## Introduction

The JANUS project was started in 1973 (1). It was named after the mythological god with two faces, symbolizing possibilities for joint prospective and retrospective studies in sera from cancer patients (1-3). The serum collection has been an ongoing task sponsored by the Norwegian Cancer Society. Serum sampling was terminated December 31, 1991 but will be started again in 1995. The status of the project up to 1991 was reported at a meeting in Vienna 1991 (4). The JANUS serum bank, as of October 1993, contained 424,938 serum specimens from 293,692 individuals. None of them had known cancer at the time of blood sampling. Since 1973 and until October 1993, however, about 14,000 of the blood donors have developed some type of cancer. Deep-frozen sera collected from a few months to 19 years prior to diagnosis are thus available for research. The primary aim of the

JANUS project is to search in these premorbid sera for chemical, biochemical, immunological, or other changes that might indicate cancer development at early stages, or be indicative of increased risk of cancer (3,4).

The serum bank, however, may also be of considerable research potential in other contexts, e.g., in studies of diseases other than cancer, and in the area of human exposure assessment.

## Materials And Methods

### Serum Collection and Storage

The donors of blood to the collection come from the Red Cross Blood Center, Oslo; from the so-called Oslo-investigation on cardiovascular disease; and from several counties in Norway, including Finnmark in northern Norway, Sogn and Fjordane in western Norway, and Oppland in mid-Norway (Table 1). In the case of donors from the Red Cross Blood Center, the routine was first to collect 450 ml of blood in the usual manner. Following this blood sampling, 50 ml of blood was collected and centrifuged. About 20 ml of serum was aliquoted into 5-ml polyethylene vials and frozen locally before transportation to the main storage room where the temperature was  $-25^{\circ}\text{C}$ . The number of Red Cross blood donors in the JANUS project was

28,664. From 4000 to 6000 blood samples have been collected from this group of individuals per year. Samples from young blood donors (25-30 years of age) were collected and stored at 3- to 5-year intervals, whereas in the case of the older donors (> 40 years) samples were taken more frequently, usually at 1- to 2-year intervals. Thus, for certain individuals the serum bank may contain up to 13 consecutive blood samples collected since 1973, whereas in other cases from one to three samples are available. On average, the Red Cross Blood donors have contributed about three samples to the serum bank.

The age distribution of the donors shows that the Red Cross donors now are from 24 to 86 years of age, whereas the

**Table 1.** Number of serum donors and samples in the JANUS serum bank.

Collection site	Number of donors	Number of specimens
Finnmark County	18,000	29,864
Oslo-investigation (men only)	17,647	17,647
Sogn og Fjordane County	19,000	33,595
Oppland County	33,000	88,808
Other counties in Norway	176,881	176,881
Radium-hospital (Oslo)	500	500
Red Cross blood donors	28,664	77,643
Total collection	293,692	424,938

This paper was presented at the Conference on Human Tissue Monitoring and Specimen Banking: Opportunities for Exposure Assessment, Risk Assessment, and Epidemiologic Research held 30 March - 1 April 1993 in Research Triangle Park, North Carolina.

Address correspondence to Dr. Egil Jellum, Institute of Clinical Biochemistry, Rikshospitalet, 0027 Oslo 1, Norway. Telephone 47 22 867035. Fax 47 22 867029.

majority of all the other donors are 52 to 72 years of age.

Samples contributed from the various counties in Norway have been drawn from persons undergoing routine health examinations, particularly in connection with evaluation of risk factors for cardiovascular disease (5). A considerably smaller volume of serum (1–10 ml) is available from these groups of donors, and one to a maximum of three consecutive samples have been collected.

Sera from the Red Cross Blood Center (drawn in conjunction with ordinary blood donations) have been handled and aliquoted specifically for the JANUS collection. The lag time between blood sampling and freezing was kept as short as possible. The quality of the Red Cross sera is therefore better than the left-over sera obtained (after lipid analysis) from the other groups of donors. In the latter cases the lag time before freezing was longer and these sera may have been kept at 4°C for a day or two before freezing.

It is also important to know that the collection of samples was divided in two phases. During the first phase, 1973 to 1987, blood was obtained at intervals from approximately 102,000 donors. After 1987 and up to the end of 1991, sera from an additional 191,000 persons were included, making up the total of 293,000 donors.

### Cancer Follow-up

Since 1951 all new cancer cases in Norway have been registered by the Cancer Registry of Norway. This compilation is based on compulsory reporting by hospital departments and histopathologic laboratories, and covers the total population (4.5 million inhabitants) of Norway. In 1960 all inhabitants of this country were given individual identification numbers (11 digits), and since that time all newborns and immigrants to Norway are systematically given such numbers. All blood donors to the JANUS collection consequently have identification numbers and these are regularly (e.g., once a year) linked with the data file of the Cancer Registry. The following numbers illustrate the cumulative cancer cases in the Janus material, based on the initial 102,000 donors: 1976, 250 cases; 1983, 1800 cases; 1986, 2700 cases; 1990, 4300 cases; autumn 1991, 5000 cases; and spring 1993, about 6000 cases. In addition there are about 8000 cumulative cancer cases among the 192,000 donors added to the collection since 1987. Thus the total cumulative number of cancers among the 293,000 donors is about 14,000 cases,

expected to rise to almost 40,000 cases by the year 2000. The rapidly increasing number of cancer cases obviously reflects the increased risk of cancer with advancing age of the donors. The dominant malignancies are skin, breast, lung, and colon cancer.

Further information on each case, including date of cancer diagnosis, type of cancer, histopathologic diagnosis, and stage and localization of the disease can readily be obtained from the Cancer Registry.

### Sample Retrieval

The serum samples are stored inside a large, commercially operated cold storage plant. The temperature is –25°C. The samples are placed in square boxes (cartons) containing 100 samples. These cartons are contained in large cardboard boxes which again are placed on removable benches. These benches may be selected by means of the plant's data system and retrieved by trucks. Each vial is labeled with date of blood sampling and identification number. The box number and the position of each sample in the small box with 100 samples have been catalogued and can easily be retrieved by our own data system. The samples to be analyzed are manually picked out, thawed and aliquoted before refreezing. In general two to three age-, sex- and storage-matched control specimens (i.e., from JANUS donors who have not yet developed cancer) are retrieved for every patient sample. All samples are given a new and internal code before shipment at –70°C (surrounded by solid carbon dioxide) to the collaborating laboratories. Because the analyses are "blind" and because the code is not broken until after completion of the analyses, bias is avoided. Moreover, the coding ensures a high degree of confidentiality, and patient names and birthday numbers are not disclosed.

### Stability of the Frozen Serum Specimens

This important question has been approached in several ways. High resolution two-dimensional (2D) protein electrophoresis has been carried out on series of serum specimens from the same blood donors, with up to 10 years difference in storage time between early and late samples. The 2D-protein patterns were surprisingly constant over the years, with only minor changes (6,7). A similar conclusion has been reached by Tracy et al. (8). It should be emphasized, however, that the 2D-analyses were carried out in the presence of reducing and denaturing agents

(mercaptoethanol and 8 M urea). The above results therefore indicate that the primary structure of the proteins withstands storage at –25°C quite well, but do not allow conclusions concerning the stability of the tertiary structure and biological activities of the serum proteins. Undoubtedly, the activity of certain enzymes will gradually be lost, whereas other enzymes are reasonably stable for many years.

With regard to immunologic activities (e.g., antibodies and antigens), the situation is more favorable than in the case of enzymes, as the immunologic properties often may be related to the primary protein structure. The HIV antibody, for example, appears to be stable for several years under our storage conditions. Thus, two donors belonging to the AIDS risk group had donated blood for several years and their serum samples were stored as usual. Retrospective antibody testing could trace positive results back to 1981, comparable with the results obtained in 1985. After 1985, HIV antibody testing of blood donors has become routine in Norway and no further HIV positive donors have been found in the JANUS collection. Also antigens, at least some of them, appear to be stable. Thus the CA-125 antigen found in serum from patients with ovarian cancer (9) and detected by the OC-125 monoclonal antibody appear to withstand storage at –25°C.

Many metabolites of low molecular weight, e.g., several organic acids, amino acids, and carbohydrates are reasonably stable in serum at –25°C. This has been demonstrated by using sensitive capillary gas-chromatography mass-spectrometry methods in our laboratory. Trace metals in serum are stable almost indefinitely, and many inorganic salts are also very stable. Lipoproteins, certain vitamins (e.g., C and E), certain oligosaccharides as well as easily oxidizable serum constituents are likely to be less stable. Polyunsaturated fatty acids, with up to six double bonds of both the omega-3 and omega-6 type, have been found (using capillary gas chromatography) to be surprisingly stable under our storage conditions.

Although our storage condition at –25°C is not ideal (liquid nitrogen temperature of –190°C, or ultra freezer at –80°C would obviously be better), one should keep in mind that even if certain serum constituents are degraded slowly at –25°C, in some cases a thorough use of matched controls can partly compensate for this change.

## Results

### Search for Cancer Markers in the Premorbid Sera

During the early years of the JANUS project, two principally different analytical approaches were taken. Multicomponent analyses, particularly using high resolution two-dimensional electrophoresis, were used to monitor changes in the pattern of several hundred serum proteins (polypeptides). Single component analyses were aimed at the determination of specific antigens, enzymes, isoenzymes, hormones, vira, or other "markers" assumed to be associated with cancer. Because the policy of the JANUS committee has been to make the serum bank available for collaborative research, several projects have been initiated both in Norway and other countries. Some of these projects were discontinued after some time because of negative results. These include, without going into any detail, analyses in the premorbid sera of compounds such as ectopic hormones (ACTH, HCG, and calcitonin) anti-T and anti-I titer (a special assay developed by Abbott Laboratories, Chicago), galactosyl-transferase isoenzyme II, and certain lung and breast cancer antigens.

Direct detection of alterations in the serum protein pattern by means of 2D-electrophoresis has also been abandoned, due to the difficulty in detecting trace proteins in the presence of major serum proteins such as albumin, immunoglobulins, etc. (6).

Other projects have given results that have been published recently. Briefly these include the following:

**Lung Cancer Antigen.** An immune antitumor factor in lung cancer, isolated by a group at the Norwegian Radium Hospital, but not characterized, gave positive test results several months prior to the clinical recognition of lung cancer (9). This was the first study in which the JANUS bank was used.

**Ovarian Cancer Antigen — CA 125.** To investigate the sensitivity of the CA 125 immunoradiometric assay for occult ovarian neoplasia, serum CA 125 levels were determined retrospectively in double-blind fashion for specimens collected from 105 women who subsequently developed ovarian neoplasia, and from 323 matched controls. The distribution of CA 125 levels was very different between the case and control populations ( $p = 0.0001$ ) over the entire collection-to-diagnosis interval (range 1–143 months). Median CA 125 levels for all cases, and for those collected

more than 24 or 36 months prior to diagnosis, were always in excess of 18 U/ml compared with a median of 10.9 U/ml for controls. Half of the cases collected within 18 months prior to a diagnosis had CA 125 levels greater than 30 U/ml and one third had levels greater than 65 U/ml. About one fourth of those collected prior to 60 months before diagnosis had levels above 30 U/ml. In contrast, approximately 7% and 0.9% of controls had levels in excess of 30 or 65 U/ml, respectively. Elevations occurred in cases eventually diagnosed with localized or advanced cancer, borderline or frankly malignant disease, but rarely in cases with mucinous neoplasms.

These results provide an insight into the preclinical biology of ovarian neoplasia that may help in designing methods for early detection or prevention of this disease, and demonstrate the usefulness of the JANUS serum bank as a resource in evaluating serum tests (10).

**Selenium, and Copper, and Thyroid Cancer.** Sera from 43 persons who developed thyroid cancer on an average 4.8 years after blood sampling were compared with sera from 129 controls, matched for sex, age, place of residence, and year of blood sampling, with regard to S-Se and S-Cu. Cases were significantly lower in S-Se than controls, and the relative risk of thyroid cancer increased from 1 for levels  $>1.60$   $\mu\text{mole/l}$ , to 7.2 for levels 1.20–1.59  $\mu\text{mole/l}$  to 15.8 for levels  $<1.20$   $\mu\text{mole/l}$ . Regression analysis showed that the S-Se concentration decreases the closer a case comes to the time of diagnosis. The relative risk estimates changed negligibly, however, when adjusted for this confounder. It is uncertain whether this adjustment leads to an erroneous conclusion about the cancer-protective effect of Se, since estimates of relative risk in the early, prediagnostic phase of thyroid cancer give no solid indications of a primary preventive effect of selenium on thyroid cancer. There was no difference between cases and controls with regard to S-Cu (11).

**Hodgkin's Disease and Epstein-Barr Virus (EBV).** This was a joint study between several universities and hospitals in the United States and several serum banks (12). The findings of this joint study indicate that elevated EBV antibodies are risk factors for the development of Hodgkin's disease. The results suggest that chronic EBV reactivation occurs several years preceding diagnosis (12).

**Serum Thyroglobulin and Thyroid Cancer.** Serum samples taken several years prior to diagnosis of cancer were analyzed

for thyroglobulin (TG) and thyroid stimulating hormone (TSH). It was found that 80% of those persons having greatly elevated serum thyroglobulin belonged to the group (43 blood donors) that later developed thyroid cancer. Calculations showed that the relative risk of thyroid cancer increases with increasing levels of TG in serum. There were no statistical differences in TSH between the cases and the controls (13).

**Polyunsaturated Fatty Acids in Serum Phospholipids and Risk of Breast Cancer.** Sera were analyzed from 87 women who developed breast cancer (cases) subsequent to blood donation and sera from 235 women who were free of any diagnosed cancer (controls), but were of similar age and had similar storage time as the cases. HPLC was used to isolate the phospholipid fraction, which was trans-methylated before determination of the fatty acid profile by capillary GC (14). The results showed that there was an inverse relation between linoleic acid (C18:2, n-6) and risk of breast cancer, but this association was restricted to women who were 55 years and younger. No similar "protective" effect was observed with n-3 unsaturated fatty acids. None of the measured fatty acids (saturated or unsaturated) showed a positive association with breast cancer risk (14).

**Prostate-specific Antigen (PSA) and Prostate Cancer.** This study comprised samples from 31 cases and 62 matched controls. A two-site immunoradiometric assay (IRMA) was used based on monoclonal antibodies against PSA and magnetizable polymer particles as the solid phase. The results show a significant difference in PSA levels between the case group and the control group, leading to an increased probability of later contracting prostate cancer if the PSA value exceeds 4 mg/l. The results support the view that elevated serum PSA identifies patients at high risk, and that this cancer can be detected by PSA measurement years before clinical diagnosis (15).

## Discussion

The JANUS serum bank with systematized serum collection between 1973 and 1992, has grown to become one of the largest banks of sera currently known (16,17). The collection is available for collaborative research with early detection of cancer as the main goal. With the rapidly increasing number of cancer cases among the (aging) donors to the JANUS project, the scientific value of the serum bank will also increase

in the years ahead. The JANUS idea (1) was initiated as a retrospective/prospective cancer study. The combination of the large serum bank with the detailed information obtainable from the Norwegian Cancer Registry makes the JANUS project especially suited for such studies. Although clinical and histopathologic data on the cancer cases are easily retrievable, there are unfortunately only limited data available on all those donors who have not developed cancer that serve as controls. In the future we hope to include medical records and mortality data on all donors in the JANUS serum bank.

Although the JANUS project so far has been devoted to cancer research, the serum

bank is in principle useful for other purposes. Among the 293,000 persons who have donated blood to the serum bank, many have contracted diseases other than cancer, e.g., heart disease, rheumatic disease, etc. Samples are available from all these persons, although the retrieval of the specimens will be more difficult because of lack of suitable registries.

One should also consider that the large serum bank might be used in environmental studies, e.g., in the area of human exposure assessment. Valuable information might be obtained by analyzing sequential samples taken between 1973 and December 1991, searching for chemical substances in the sera that might reflect

differences in exposure to environmental pollutants in this 19-year period. Until now, however, our serum bank has not been used for such purposes, but the steering committee of the JANUS bank is open to suggestions.

## Conclusion

A large serum bank containing close to 500,000 serum samples collected from 293,000 persons over a 19-year period is available for research purposes. So far the bank has been used on cancer-related problems, but the serum collection has an obvious potential also environmental studies.

## REFERENCES

1. Torgersen O. A possibility for some joint prospective and retrospective studies in sera from cancer patients. In: Excerpta Medica, Intern. Congress Series 275 (Maltoni C, Crepsi M, Burch PRJ, eds). Amsterdam:Elsevier, 1973;121.
2. Ørjasæter H. The JANUS serum research programme: a multipurpose project for retrospective as well as prospective investigations of cancer. *Acta Path Microbiol Scand Sec. A, Suppl* 248:175–180 (1974).
3. Jellum E, Andersen A, Ørjasæter H, Foss OP, Theodorsen L, Lund-Larsen P. The JANUS serum bank and early detection of cancer. *Biochim Clinica* 11:191–195 (1987).
4. Jellum E, Andersen A, Lund-Larsen P, Theodorsen L, Ørjasæter H. The JANUS serum bank. *Sci Tot Environ* 139/140:527–535 (1993).
5. Bjartveit K, Foss OP, Gjervig T, Lund-Larsen P. The cardiovascular disease study in Norwegian counties. *Acta Med Scand Suppl* 6:34 (1979).
6. Jellum E, Thorsrud AK. Clinical applications of high resolution two-dimensional electrophoresis. *Clin Chem* 28:876–883 (1982).
7. Jellum E, Thorsrud AK, Vatn M, Grimstad I, Brennhovd I, Tveit KM, Pihl A. Detection of cancer-related proteins by two-dimensional electrophoresis. *Ann NY Acad Sci* 428:173–185 (1984).
8. Tracy RP, Currie R, Young DS. Two-dimensional gel electrophoresis of serum specimens from a normal population. *Clin Chem* 28:890–899 (1982).
9. Kotlar HK, Sanner T, Eker P, Pihl A, Ørjasæter H, Harvei S, Theodorsen L, Jellum E. Anti-tumor response in the preclinical period of lung cancer. *Eur J Cancer Clin Oncol* 18:317–319 (1982).
10. Zurawski VR, Ørjasæter H, Andersen A, Jellum E. Elevated serum CA 125 levels prior to diagnosis of ovarian neoplasia: relevance for early detection of ovarian cancer. *Intern J Cancer* 42:677–680 (1988).
11. Glattre E, Thomassen Y, Thoresen SO, Lund-Larsen P, Theodorsen L, Aaseth J. Prediagnostic serum selenium in a case-control study of thyroid cancer. *Int J Epidemiol* 18:45–49 (1989).
12. Mueller N, Evans A, Harris N, Comstock GW, Jellum E, Magnus K, Orentreich N, PolkVogelman J. Hodgkin's disease and Epstein-Barr virus: altered antibody pattern before diagnosis. *N Engl J Med* 320:689–695 (1989).
13. Thoresen SO, Myking O, Glattre E, Røotwelt K, Andersen A, Foss OP. Serum thyroglobuline as a preclinical tumor marker in subgroups of thyroid cancer. *Br J Cancer* 57:105–108 (1988).
14. Vatten LJ, Bjerve KS, Andersen A, Jellum E. Polyunsaturated fatty acids in serum phospholipids and risk of breast cancer: a case-control study from the JANUS serum bank in Norway. *Eur J Cancer* 29A:532–539 (1993).
15. Paus E, Theodorsen L, Engeland E. Prostate-specific antigen in serum from blood donors with subsequent cancer prostate diagnosis. *Eur J Cancer* 1993 (in press).
16. Biological Materials Banks, Clearing-House for On-going Research in Cancer Epidemiology, International Agency for Research on Cancer, Lyon:International Agency for Research on Cancer, 1985.
17. Coleman M, Wahrendorf J, eds. Directory of On-going Research in Cancer Epidemiology 1991. IARC Scientific Publ no 110, Lyon:International Agency for Research on Cancer, 1991.